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A New Apparatus for Separation of Chemical Compounds by Liquid-Liquid Extraction

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Partition between two immiscible or partially miscible solvents has in recent years become a very valuable tool for the isolation, determination and characterization of chemical substances. The first apparatus for "countercurrent" extraction was the one described by Jantzen [1]. Other workers have subsequently built apparatus on the same principle. The first laboratory-scale device with continuous flow of solvents was the column of Cornish *et al.* [2] The excellent work of Craig and his associates [3,4] has made the counter-current distribution technique a useful routine method. Another apparatus working on the same principle is that described by v. Metzsch [5]. For the large number of other works reference should be made to more comprehensive reviews on the subject [6,7].

In this laboratory there has been a need for methods for the isolation, determination and characterization of different chemical substances of plant origin. For this purpose I have developed a new type of counter-current apparatus with 100 distribution tubes, using continuous solvent flow. This may be regarded as an improved modification of an apparatus developed by Weygand *et al.* [8] The new apparatus is intended for the separation of one substance from a mixture by solvent distribution. It has the following advantages:

1) The construction is extremely simple. There are no complicated mechanical or glass parts. This makes the operation very reliable. The building of the apparatus is not very costly.

2) The apparatus works precisely according to theoretical calculations. This has usually not been true of earlier apparatus with continuous solvent flow, which has been a serious objection by comparison with apparatus functioning after the "decanting" principle.

3) There are no limiting factors in the use of different solvent systems. The apparatus has been loaded with large amounts of raw unpurified plant extracts without stable emulsions being produced.

4) The apparatus has 100 tubes, but in principle the number of tubes is unlimited.

5) It is possible to run the apparatus with either flowing upper phase or flowing lower phase, these flowing in opposite directions. This can be done with successively smaller amounts of both phases, which increases considerably the effective number of tubes in the isolation of a substance. In this way the apparatus corresponds theoretically to about 1 100 distribution tubes without recycling for a 99.7 % yield and approximately 2 500 tubes for a 95.5 % yield. In practice, distributions corresponding to some hundred "transfers" can be conveniently made.

An important defect is that work with this apparatus is somewhat more time-consuming than with apparatus using the "decanting" principle.

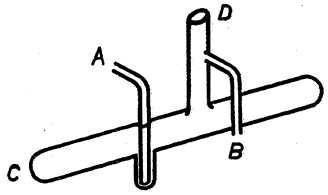

Fig. 1. See text.

The design of one distribution unit is seen in Fig. 1. Tube A is the inlet tube for the upper phase, tube B corresponds to tube A in the next cell. Ten such units are fused together into one piece, and these are connected to each other with suitable plastic tubing. In runs where lower phase flows in the reverse direction, it enters through tube B and leaves the unit through A. The equilibration is made by a continuous swinging movement of the main tube C in its length-wise direction through an angle of $\pm 30°$. The tubes are filled through D. Tubes D are closed with stoppers. In the two end tubes there are stopcocks for changing the solvent and the direction of flow. The whole row of 100 tubes is mounted on a suitable bar and made to swing by an electric motor. The frequency and amplitude of the strokes can be adjusted. The flow rate of solvents is controlled with Mariotte's flasks at both ends of the apparatus. The apparatus is used with a fraction collector having a moving center arm. The fraction collector is shifted from one end to the other if the solvent and direction of flow are changed.

Table 1. Distribution constants and recoveries in the distribution of oxalic, succinic and glutaric acid mixture presented in Fig. 2.

Acid	K, determined in separatory funnels	Conc. in the upper phase, normality	K, calc. from distr. curves	Substance weighed mequiv.	Substance found from distribution	Recovery %
Oxalic	0.24—0.22	0.02—0.002	0.23	5.85	5.89	100.8
Succinic	1.09—1.02	0.05—0.004	1.08	4.95	4.91	99.3
Glutaric	2.05—1.80	0.06—0.002	1.90	4.91	4.96	101

The volume of one tube is 86 ml. The relative amounts of the upper and lower phases can be varied over at least the ratios 3—0.33.

The theory of the apparatus cannot be presented here in detail. Stene [9] *e. g.* has made theoretical calculations concerning distribution in apparatus functioning after this principle. The distribution, presented as a function of tube number, of the mass of a solute A introduced into the first tube at the beginning of the distribution, forms a Poisson distribution curve known from statistics. When the tube number is large, it can be approximated by a standard error curve. In this apparatus the standard deviation δ of the normal error curve is $\delta = \sqrt{x}$, where x is the number of those tubes through which the maximum of the solute A has passed. When the solute A has passed through the whole apparatus and has been collected in fractions, the amount of effluent corresponding to δ is approximately $\sqrt{x(v + KV)}$, where v is the amount of the flowing phase and V the amount of the stationary phase in one distribution unit. K is the distribution coefficient of the substance.

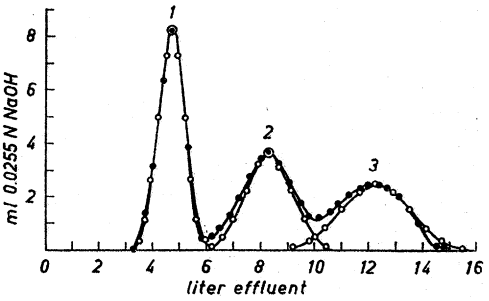

Fig. 2. Distribution of oxalic, succinic and glutaric acids in a solvent system *n*-butanol-water at 20°C. Run in one direction only with the lower phase flowing. Volume ratio 1:1. Flow speed 6 ml/min. ●———● experimental curve by titration O———O calculated curves, 1, oxalic acid, 2, succinic acid, 3, glutaric acid.

Fig. 2 shows the distribution of a known mixture of oxalic, succinic and glutaric acids in the solvent system *n*-butanol-water. These acids are known to have distribution coefficients which are relatively concentration-independent. Table 1 shows numerical values concerning the distribution.

As an example of the fractionation of substances of biological origin Fig. 3 shows distribution of three sulfur-containing glucosides from turnip seeds.

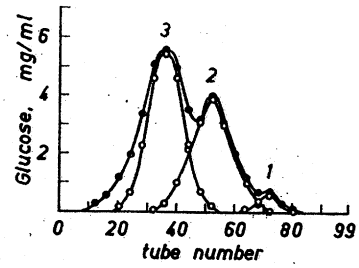

Fig. 3. The distribution of three sulfur-containing glucosides from turnip seeds in a solvent system *n*-butanol-ethanol-water (4:1:4) at 20°C. Volume ratio 46:40. The points of the curve represent samples from the tubes of the apparatus after 22.5 liters of the upper phase had flown through with a speed of 10—15 ml/min. Ordinate: Glucose as determined quantitatively by the anthron method. Abscissa: Tube number. ●———● experimental curve. Curve 1: Gluconapin, K 0.148; Curve 2: Gluconasturtin, K 0.104; Curve 3: Progoitrin, K 0.066. The deviation of the last curve depends on dissociation-association phenomena of the last substance in the unbuffered solvent system. The whole amount of substance was about 8 g dry weight.

The exact mathematical and theoretical treatment and technical details omitted from this preliminary note will be presented in a further paper.

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